

# Isotopic Tracing of Nitrogen Sources and Cycling in the Mississippi River Basin

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## ABSTRACT

As part of five pilot studies, several different isotopic approaches for tracing nutrient sources, land uses, and nitrogen cycling processes that are contributing nitrogen to the Gulf of Mexico are being evaluated. Analysis of nitrate for both  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  enhances our ability to distinguish between variations in  $\delta^{15}\text{N}$  due to mixing of sources and variations due to denitrification. Analysis of organic matter for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  provides information on the terrestrial versus aquatic origin of the material. The  $\delta^{15}\text{N}$  of fish collected at large-river are evaluated as integrators of local variability in nitrogen sources. Preliminary data from these fish suggest that source signatures are being substantially overprinted by other processes at these sites.

## INTRODUCTION

High nitrate levels in streamwater in the Mississippi River Basin pose potential problems for water supplies in the region, affect local ecosystem health, and are suspected of causing a large hypoxic zone in the Gulf of Mexico each summer. We are investigating several different isotopic approaches for tracing nutrient sources, land uses, and nitrogen (N) cycling processes that are contributing N to the Gulf of Mexico. In particular, we are evaluating the relative merits of measurements of the nitrogen and oxygen isotopic compositions of nitrate, and the carbon, nitrogen, and sulfur isotopic compositions of particulate organic matter (POM), dissolved organic matter (DOM), algae, fish, and invertebrates as environmental indicators.

## STUDY DESIGN

Five pilot studies are in progress with stream samples collected as part of the U. S. Geological Survey's National Stream Quality Accounting Network (NASQAN) and National Water Quality Assessment (NAWQA) monitoring programs, and at Biomonitoring of Environmental

Status and Trends (BEST) and National Contaminant Biomonitoring Program (NCBP) sites. To date, only about half of the collected samples have been analyzed for stable isotopes.

Nitrate samples were collected on exchange resins and analyzed for stable isotopic composition using the Silva and Chang methods described in Kendall (1998). The nitrogen and oxygen stable isotopic compositions (denoted  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ , respectively) of nitrate are reported in units of permil (parts-per-thousand, denoted ‰) relative to the reference standards Air and V-SMOW, respectively. Organic samples were dried, homogenized, combusted, and analyzed by continuous-flow mass spectrometry. The carbon, nitrogen, and sulfur stable isotopic compositions (denoted  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$ , respectively) of organic samples are reported in permil relative to the reference standards V-PDB, Air, and Cañon Diablo Troilite, respectively.

Below are descriptions of the types of samples collected for the 5 pilot studies:

1. Samples were collected during selected periods in 1996-98 from 7 sites on major tributaries, and at 17 sites on smaller rivers draining basins dominated by either row crop

agriculture, animal production, urban, or undeveloped land uses (Battaglin and others, 1997). Nitrate was analyzed for  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$ . Particulate organic matter (POM) and dissolved organic matter (DOM) were analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , with a subset analyzed for  $\delta^{34}\text{S}$ .

2. Fish samples collected in 1986 and 1995 from some 50 locations within the Mississippi basin (and about 120 sites elsewhere in the USA) were analyzed for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$ . We investigated the relations among total nitrogen concentration in the water, the  $\delta^{15}\text{N}$  value of biota, and watershed land use, using the isotopic compositions of fish as integrators of the isotopic signal of riverine biota. Fish representing two trophic levels were collected and analyzed: invertebrate-eating fish (mainly carp and catostomids) and fish-eating fish (several species of bass).
3. POM samples were collected biweekly to monthly for over a year (1996-98) from some 15 sites in the basin (and at the rest of the NASQAN sites) and analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ; a subset were analyzed for  $\delta^{34}\text{S}$ . The isotopic compositions of POM provide information about the sources of the particulates (e.g., terrestrial detritus versus *in situ* production in the water column).
4. Intersite isotopic variability in POM and benthic algae (periphyton) samples was assessed by a NAWQA synoptic study conducted during August 1997 along 70 smaller tributaries in the basin. All samples were analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ; a subset were analyzed for  $\delta^{34}\text{S}$ .
5. Archived fish and invertebrate samples collected at selected NAWQA indicator sites (moderate-sized basins with homogeneous land use) from mid-1995 through 1998 are being analyzed now for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  to better understand the relations between land use and the isotopic compositions of prospective environmental isotopic indicators and integrators.

## THEORETICAL BASIS

Many studies have shown that nitrate isotopes can be effective tools for distinguishing among potential sources of N in agricultural areas (Kendall, 1998). In particular, fertilizer and animal waste (including human waste) have very different  $\delta^{15}\text{N}$  values ( $0 \pm 2\text{‰}$  versus  $+15 \pm 5\text{‰}$ ), so that the relative contributions from these sources can often be estimated by measuring the  $\delta^{15}\text{N}$  of the nitrate in streams or groundwater. However, biogeochemical recycling of nitrate (especially denitrification) can cause large changes in  $\delta^{15}\text{N}$ , making such estimates problematic.

The first attempt to use  $\delta^{15}\text{N}$  of nitrate to estimate sources of nitrate in major streams in the Mississippi basin (Kohl and others, 1971) elicited such a storm of protest from the soil science community (Hauck and others, 1972) that it has not been attempted again until now. Most of the criticism was aimed at the difficulty of determining source information from the  $\delta^{15}\text{N}$  values of stream nitrate when the nitrate has been affected by denitrification. Analysis of nitrate for both  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  provides an enhanced ability to distinguish mixing from denitrification.

The theoretical basis of the "biomass isotope" studies is that the isotopic compositions of plankton and non-N fixing plants mainly reflect, to a large extent, the isotopic compositions of the dissolved N, C, and S in the environment that is being utilized by the biota and subsequently modified by various possible fractionating mechanisms in the plants. The isotopic compositions of the primary producers are then reflected by higher level organisms such as invertebrates and fish, as modified by mixed diets, trophic enrichments, and temporal/spatial variability in feeding behaviors. Hence, the  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values of plants and organisms in the environment will reflect the interplay of "source" signatures (e.g., from agriculture, livestock, urban, atmospheric, soil), geochemical "process" signatures (e.g., resulting from denitrification, methane oxidation, sulfate reduction), and biochemical metabolic and catabolic fractionations (e.g., trophic enrichments, turnover rates, etc).

## RESULTS AND DISCUSSION

Nitrate samples collected during the early spring through summer (the fall samples are currently being analyzed) show a 16‰ range in  $\delta^{15}\text{N}$  and a 20‰ range in  $\delta^{18}\text{O}$ . Low-nitrate urban and undeveloped areas generally show the highest  $\delta^{18}\text{O}$  and lowest  $\delta^{15}\text{N}$  values, with values in the range expected for atmospheric nitrate and nitrate fertilizers. Otherwise, most other samples have  $\delta^{15}\text{N}$  values that fall within the ranges expected for microbial nitrate derived from ammonium fertilizer, soil, and manure nitrogen sources.

The classic way to distinguish between mixing and recycling processes as major controls on the  $\delta$  values and nitrate ( $\text{NO}_3$ ) concentrations is to make plots of the  $\delta$  values versus  $1/\text{NO}_3$  and  $\ln \text{NO}_3$ . Linear trends on such diagrams indicate mixing and recycling, respectively, because mixing relations are hyperbolic and denitrification causes an exponential increase in  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  with decreasing nitrate concentrations (Kendall, 1998). Preliminary evaluation of data indicates substantial seasonal variations in  $\delta$  values at most NAWQA indicator sites. The variability at some sites appears to be controlled mainly by mixing, but others show complicated mixtures of processes. Comparison of the nitrate data with other isotope data from POM or fish samples may help constrain the extent of possible riverine recycling of nutrients.

Preliminary  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  values for nitrate from large river (NASQAN) sites show that the values for each site in the upper Mississippi basin (Mississippi River at Clinton, Ohio River near Grand Chain, and Missouri River at Hermann) appear to cluster in distinct areas, and have a larger variation in  $\delta^{18}\text{O}$  than in  $\delta^{15}\text{N}$ . Samples from Mississippi River at Thebes, which are a mixture of water from Clinton and Hermann, exhibit a large shift to lighter  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  values, possibly indicating the input of fertilizer from the basins in Eastern Iowa and Illinois that flow into the Mississippi between Clinton and Thebes. Samples from the Mississippi River at St. Francisville lie between those from Thebes and Grand Chain, as expected. More data will be required to confirm these observed patterns.

Samples collected from the Mississippi River at Thebes (MRTB) on 4/16/98, the Ohio

River at Grand Chain (ORGC) on 4/23/98, and the Mississippi River at St. Francisville (MRSF) on 4/30/98 constitute a nearly Lagrangian sample set. The discharge, chloride load,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  of the MRSF samples are consistent with simple mixing of the MRTB and ORGC waters. The combined discharge associated with the MRTB and ORGC samples is 103% of the discharge associated with the MRSF sample, and the chloride load calculated from these two samples is 92% of the chloride load of the MRSF sample. The  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values predicted by mixing MRTB and ORGC water (+6.5‰ and +8.8‰, respectively) are very close to the values measured in the MRSF sample (+6.5‰ and +8.3‰, respectively). However, the nitrate and total N loads for the MRSF sample (6,070 and 7,495 tonnes/day) are only 83% of the sum of the nitrate loads and 70% of the sum of the total N loads in the MRTB and ORGC samples.

The mechanism for this loss of N is unclear. If the missing nitrate had been lost via progressive denitrification along the river reach between Grand Chain and St. Francisville, the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values of the remaining nitrate should have increased; however, there is no evidence for this in the isotopic compositions. Other possible processes that could account for the observed chemical and isotopic data include assimilation by aquatic plants (a process that generally causes minimal fractionation) or dilution by mixing with groundwater with a lower nitrate concentration but roughly similar isotopic composition. This analysis could be improved if samples were periodically collected from NASQAN stations using a Lagrangian strategy.

The C/N of the POM collected at NASQAN sites was generally in the range of 5 to 10, indicating that very little of the POM is terrestrial in origin; hence the isotopic compositions reflect processes in the water column. The  $\delta^{13}\text{C}$  values ranged from -35 to -17‰, with seasonal variability ranging from 3 to 13‰. The  $\delta^{15}\text{N}$  values ranged from +1 to +12‰, with seasonal variability ranging from a low of 3‰ (e.g., Missouri River at Hermann) to a high of 8‰ (e.g., Tennessee River at Paducah). Several sites showed a progressive increase in  $\delta^{15}\text{N}$  over the year. The  $\delta^{15}\text{N}$  values of POM showed the same general range of values as nitrate, indicating that

assimilation of nitrate caused minimal fractionation.

The  $\delta^{15}\text{N}$  of invertebrate-feeding fish (mostly carp and catostomids) which varied from +9 to +18‰ among sites, was significantly ( $P < 0.01$ ) related to total N. A similar relation was found for bass, but the  $\delta^{15}\text{N}$  values were higher because of their higher trophic position. Preliminary statistical analysis showed little correlation of  $\delta^{15}\text{N}$  with land use. However, there was a good positive relation of  $\delta^{15}\text{N}$  of invertebrate-feeding fish and the percent of the watershed “exploited” by urbanization and agriculture.

The observation that fish from watersheds dominated by agriculture and fish from watersheds dominated by urbanization both had high  $\delta^{15}\text{N}$  values is surprising. We had expected that the fish from agricultural areas would have lower  $\delta^{15}\text{N}$  values because of the lower  $\delta^{15}\text{N}$  values of the applied fertilizer. The absence of a fertilizer “signal” suggests a greater degree of recycling of the fertilizer N in large watersheds than anticipated. Hence, the  $\delta^{15}\text{N}$  may not be an effective indicator of the source of nitrogen (fertilizer versus sewage) in large watersheds, but instead an indicator of the extent of N-fractionating processes such as denitrification and volatilization that accompany excess nitrogen loadings.

Since  $\delta^{15}\text{N}$  has proved to be such an effective indicator of sources in smaller streams, we are now attempting to use archived fish samples from smaller watersheds of variable land use to determine at what scale the source signatures become “overprinted” by post-depositional biogeochemical recycling. NAWQA indicator sites, because they were chosen to have known, moderately homogeneous land uses (agricultural, urban, feedlot, and undeveloped), are an ideal “landscape-scale” set of sites to test whether the isotopic compositions of aquatic organisms collected in medium to large watersheds retain sufficient “source signal” to still provide land-use information.

## SUMMARY

Preliminary data from our 5 pilot studies (only about half of the planned samples have been analyzed thusfar for stable isotopes) show encouraging signs that some of these isotope approaches will provide useful new information about sources and cycling of nutrients in the Mississippi Basin. Analysis of nitrate for both O and N isotopes will significantly improve our ability to distinguish between variations in  $\delta^{15}\text{N}$  caused by mixing of sources versus denitrification. POM appears to be mainly autochthonous and the  $\delta^{15}\text{N}$  values suggest minimal fractionation of nitrate during assimilation in the big-river sites. The  $\delta^{15}\text{N}$  values of fish collected at large-river sites suggest that source signatures are being substantially overprinted by other processes at these sites.

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